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Human tissue kallikrein gene delivery attenuates hypertension, renal injury, and cardiac remodeling in chronic renal failure

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Human tissue kallikrein gene delivery attenuates hypertension, renal injury, and cardiac remodeling in chronic renal failure.

Background. Tissue kallikrein cleaves kininogen substrate to produce the potent vasodilating peptide kinin, which plays important roles in cardiovascular and renal function. To explore cardiac and renal potential protective effects of kallikrein gene delivery in chronic renal failure, we delivered adenovirus carrying the human tissue kallikrein cDNA (cHK) into rats with 5/6 reduction of renal mass.

Methods. Expression of human tissue kallikrein in rats was assessed by enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR)/Southern blotting. Physiological parameters monitored in rats included systolic blood pressure, heart rate, and urinary excretion of protein, albumin, kinin, cGMP, cAMP, and nitrate/nitrites. Systemic and regional hemodynamics were measured by fluorescent-labeled microspheres. Heart weight and myocyte diameter were used to assess left ventricular hypertrophy. Quantitative and qualitative morphological analyses were used to evaluate histologic changes in kidney and heart sections.

Results. Active tissue kallikrein reached a peak serum level of 463 ± 76 ng/mL following gene delivery and returned to control levels within 21 days. A maximal blood pressure reduction of 37 mm Hg was observed within one week in rats receiving kallikrein gene delivery as compared with control rats receiving adenovirus containing the luciferase gene (159 ± 5 vs. 196 ± 6 mm Hg, $N = 15$, $P < 0.001$), and a significant blood pressure difference continued for five weeks postgene delivery. Kallikrein gene delivery significantly decreased total urinary protein and albumin excretion and increased levels of urinary kinin, nitrite/nitrate, and cGMP levels. Cardiac output and regional blood flow were also increased, while peripheral vascular resistance decreased. Kallikrein gene transfer reduced glomerular sclerotic lesions, tubular damage, lumenal protein cast accumulation, and interstitial inflammation in the kidney. Myocardial hypertrophy and fibrosis were also attenuated in rats receiving kallikrein gene delivery.

Conclusions. These findings indicated that kallikrein gene delivery attenuates hypertension and protects against renal injury and cardiac remodeling in the rat remnant kidney model of chronic renal failure.

Key words: remnant kidney model, vasodilation, blood pressure, cardiovascular function, hemodynamics.

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Tissue kallikrein is a serine proteinase present in many tissues, including those critical for blood pressure regulation and cardiovascular function, such as the kidney, vasculature, lung, heart, and adrenals [1–3]. The best known function of tissue kallikrein is the cleavage of low molecular weight kininogen to release kinin, a peptide hormone that mediates a number of biological responses including increased local blood flow, smooth muscle relaxation or constriction, and increased vascular permeability [4]. By binding to endothelial bradykinin B_2 receptors, kinin stimulates release of potent vasodilators, including prostacyclin, nitric oxide, and endothelium-derived hyperpolarizing factor [5]. Studies have shown that hypertensive patients, as well as genetically hypertensive rats, have decreased urinary kallikrein levels [6–8]. A large family pedigree study in Utah (USA) indicated that high urinary kallikrein excretion, expressed as a dominant allele, might have a protective effect against the development of hypertension [9]. We have created transgenic animal models with altered expression of kallikrein-kinin system components in order to provide direct evidence of their role in blood pressure regulation [10–12]. Transgenic mice overexpressing human tissue kallikrein exhibited a life-long reduction in blood pressure, and this effect was shown to be mediated by the bradykinin B_2 receptor. Transgenics overexpressing human bradykinin B_2 receptors are similarly hypotensive for life. The ability of kallikrein gene delivery to lower systemic blood pressure and attenuate cardiovascular and renal injury in genetically or experimentally hypertensive rats has also been previously demonstrated [13–15].

Chronic renal failure, whether caused by diabetes, essential hypertension, chronic glomerulonephritis, or other causes, tends to progress steadily to end-stage renal disease. Systemic and glomerular hypertension and subsequent glomerular hyperfiltration are postulated to play key mechanistic roles in chronic renal failure in humans and 5/6 nephrectomized rats [16, 17]. Persistent activation in the kidney and vasculature of pressor systems, including the renin-angiotensin-aldosterone system, the sympathetic nervous system, and endothelin, are path-

ways by which hypertension arises and/or is maintained in clinical and experimental chronic renal failure [18–21]. Shimamoto et al found urinary kallikrein excretion decreased significantly in nephrectomized rats, and a negative correlation existed between urinary kallikrein level and blood pressure, indicating that a repressed renal kallikrein-kinin system may play a pathophysiological role in chronic renal failure [22]. Studies using an angiotensin-converting enzyme (ACE) inhibitor in combination with a specific bradykinin B_2 receptor antagonist (HOE140, icatibant) have shown that the renal protective effects of ACE inhibition in rat chronic renal failure models may not be dependent on kinin [23–25]; however, the issue remains controversial, as a recent study strongly implicated kinin in partially mediating these beneficial effects [26].

In the present study, we explored remedial effects of a continuous supply of human tissue kallikrein on cardiovascular and renal sequelae in 5/6 nephrectomized rats by injection of high-efficiency adenovirus harboring human tissue kallikrein cDNA. The results established a sustained blood pressure-lowering effect accompanied by improved cardiac function and lowered total peripheral vascular resistance. In addition to attenuation of renal injury and decreased proteinuria, kallikrein delivery to 5/6 nephrectomized rats reduced the development of cardiac hypertrophy and fibrosis. These findings provide important information and a possible therapeutic model for the treatment of hypertensive and chronic renal disorders.

METHODS

Preparation of replication-deficient adenoviral vectors Ad.CMV-cHK and Ad.CMV-Luc

E1/E3-deleted, replication deficient adenoviruses, in which the expression of human tissue kallikrein (Ad.CMV-cHK) or luciferase (Ad.CMV-Luc) cDNA was under control of the cytomegalovirus enhancer/promoter, were constructed as previously described [14].

Reduced renal mass model and experimental protocol

Male Wistar rats weighing between 175 and 225 g (Sprague-Dawley Harlan, Indianapolis, IN, USA) were used in this study; 5/6 renal mass reduction was performed ($N = 67$) as previously published [27], beginning with infarction of approximately two thirds of the left kidney by ligation of the posterior and one or two anterior extrarenal branches of the main renal artery. One week later, a right unilateral nephrectomy was performed. A control group (sham) of 17 rats underwent sham operations consisting of laparotomy and manipulation of the renal pedicles. Anesthesia for surgical procedures was accomplished by intraperitoneal injection of ketamine (9 mg/100 g body weight)/xylazine (1 mg/100 g body weight). All procedures complied with the standards as stated in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Resources, National Academy of Sciences, Bethesda, MD, USA). One week after surgery, 5/6 nephrectomized animals were randomly selected for the following three groups: 5/6 reduced renal mass without treatment (RRM group), rats injected via the tail vein with 1.2×10^{10} plaque-forming units of adenoviral particles harboring the gene for either human tissue kallikrein (cHK group), or luciferase (Luc group). Morphological and physiological parameters were measured in rats sacrificed at weeks 4 and 5 postgene delivery.

Blood pressure measurement and collection of serum and urine

Systolic blood pressure measurements, 24-hour urine collection, and phlebotomy for serum samples were performed as previously described [14, 15].

Expression of human tissue kallikrein in nephrectomized rats

Reverse transcription-polymerase chain reaction (RT-PCR) Southern blot analysis specific for human tissue kallikrein mRNA was performed as previously described [3]. Levels of human tissue kallikrein in serum or urine were determined by enzyme-linked immunosorbent assay (ELISA) as previously described [14, 15]. Since the antibody to human tissue kallikrein used in the ELISA only recognizes active kallikrein [14], the immunoreactive kallikrein levels represent active kallikrein.

Measurements of blood urea nitrogen and urinary kinin, albumin, NOx, cGMP, and cAMP

Serum levels of urea nitrogen were measured as previously published [28]. Urinary kinin levels were measured by a direct kinin radioimmunoassay [14]. Radioimmunossays of urinary albumin, cAMP, and cGMP levels were conducted according to previously described procedures [29, 30]. Urinary NOx content was measured by a fluorometric assay for nitrite/nitrate [31].

Fluorescent microsphere determinations of hemodynamics

Hemodynamic parameters were assessed in rats one week following gene delivery. Fluorescent microspheres with a diameter of 15 μm (Molecular Probes Inc., Eugene, OR, USA) were injected essentially as previously described [32]. A microsphere solution (0.2 mL, approximately 2×10^5 microspheres/mL in 0.9% saline + 0.02% Tween 20) was injected into the left ventricle at a rate of 0.29 mL/mine. A saline flush followed at the same rate for 140 seconds. A reference blood sample was withdrawn via the left femoral artery at a rate of 0.29 mL/min for 180 seconds starting at the same time as microsphere injection. Samples were processed by the sedimentation

method as directed by the manufacturer (Molecular Probes). Cardiac output (CO) was calculated as follows:

$$CO = Q_r \times Fluor_I \times Fluor_r^{-1}$$

where Q_r is the reference blood flow, $Fluor_I$ is total injected fluorescence, and $Fluor_r$ reference fluorescence. CO was correlated to body weight and expressed as cardiac index (CI) = CO × weight^{-1}. Total peripheral resistance index (TPRI) was calculated as mean arterial pressure (MAP) × CI^{-1}. Regional blood flow (mL/min/100 g) to tissues was calculated as = $(Fluor_{Tiss}/Wt_{Tiss})(Q_r/Fluor_r)100$.

Morphological analysis

Left ventricle weight to whole heart weight ratio was determined as previously described [15]. Sections of left ventricle were silver stained, and cardiomyocyte diameters were measured as previously published [15]. Glomerular lesions were graded in periodic acid-Schiff (PAS)-stained sections of kidney in a single-blind manner using a scale of 0 (normal) to 5 (severely sclerotic), essentially as formerly described [33]. Thirty randomly selected glomeruli were graded for each rat, and the final glomerular sclerosis score for each group was calculated from weighted values based on the percentage of glomeruli falling into each severity grade (0 through 5). Left ventricular fibrosis was assessed in low-power (×1.25 objective) fields of Masson's trichrome-stained cross-sections in Adobe PhotoShop to determine the percentage of collagen (blue) staining area to total tissue area.

Statistical analysis

Results are expressed as mean ± SEM. Comparisons among groups were made by analysis of variance with Fisher's protected least significant difference (PLSD). Differences were considered significant at $P < 0.05$.

RESULTS

Blood pressure reduction after intravenous injection of Ad.CMV-cHK adenovirus

Figure 1 shows systolic blood pressures of nephrectomized rats before and after adenovirus-mediated kallikrein gene delivery. Prior to gene delivery, the systolic blood pressure of nephrectomized rats was significantly higher (181 ± 3 mm Hg, $N = 44$, $P < 0.001$) than that of sham-operated rats (145 ± 4 mm Hg, $N = 11$). One week after adenovirus administration, a maximal blood pressure reduction of 37 mm Hg (159 ± 5 vs. 196 ± 6 mm Hg, $P < 0.001$) was observed in rats receiving the kallikrein gene (cHK, $N = 14$), as compared with rats receiving the luciferase gene (Luc, $N = 15$). Five weeks postdelivery remnant kidney rats receiving cHK ($N = 6$) maintained a 17 mm Hg (204 ± 6 vs. 226 ± 7 mm Hg, $P < 0.05$) reduction in blood pressure compared with rats receiving adenovirus containing the luciferase gene ($N = 4$, $P < 0.05$).

Expression of human tissue kallikrein after gene delivery

Expression of human tissue kallikrein mRNA in 5/6 nephrectomized rats one week following gene delivery was detected by RT-PCR followed by Southern blot analysis using oligonucleotides specific for human tissue kallikrein. Figure 2 shows that human kallikrein can be detected in the heart, aorta, lung, kidney, and liver (upper panel, left). The RT-PCR products from rats receiving the luciferase gene did not hybridize to the human tissue kallikrein gene probe (upper panel, right). These results indicate that Southern blot analysis is specific for human tissue kallikrein, and endogenous rat tissue kallikrein family members do not interfere with the assay. Similar levels of β-actin mRNA were detected in tissues of both experimental and control groups, indicating an acceptable quality and similar quantity of RNA in these samples (lower panel). Following intravenous injection of Ad.CMV-cHK into nephrectomized rats, recombinant human tissue kallikrein levels in rat sera and urine were measured by a specific ELISA. Human tissue kallikrein was detected from 3 to 14 days postgene delivery with a peak level of 463 ± 76 ng/mL on day 3 ($N = 4$ to 6). Human tissue kallikrein was detected in rat urine at 4-, 11-, and 17-days post-gene delivery (703 ± 260, 53.6 ± 27.4, 12.7 ± 12.7 ng/mL, respectively, $N = 7$). Linear displacement curves of a serial dilution of rat sera and urine from rats injected with the kallikrein gene were parallel to the purified human tissue kallikrein standard, indicating their immunologic identity (data not shown). Human tissue kallikrein was not detected in sera or urine of control rats receiving the luciferase gene. These results indicated that the antihuman tissue kallikrein antiserum did not cross-react with the members of the rat kallikrein gene family.

Ad.CMV-cHK gene delivery increases urinary kinin, NOx, and cGMP

Figure 3 demonstrates that urinary excretion of kinin, nitrate/nitrite (NOx), and cGMP were significantly increased in nephrectomized rats receiving Ad.CMV-cHK as compared with nephrectomy alone or rats receiving Ad.CMV-Luc injection. Assays were done one week following gene delivery. The data indicate that kallikrein gene delivery resulted in increased production of kinin with subsequent effects, such as lowered blood pressure, being mediated by a NO-cGMP signaling pathway. Levels of urinary cAMP did not differ significantly among experimental groups (RRM = 230 ± 24, Luc = 337 ± 92, cHK = 357 ± 153 ng/day/100 g body wt). The level of NOx is intended as an indirect measurement of NO production.

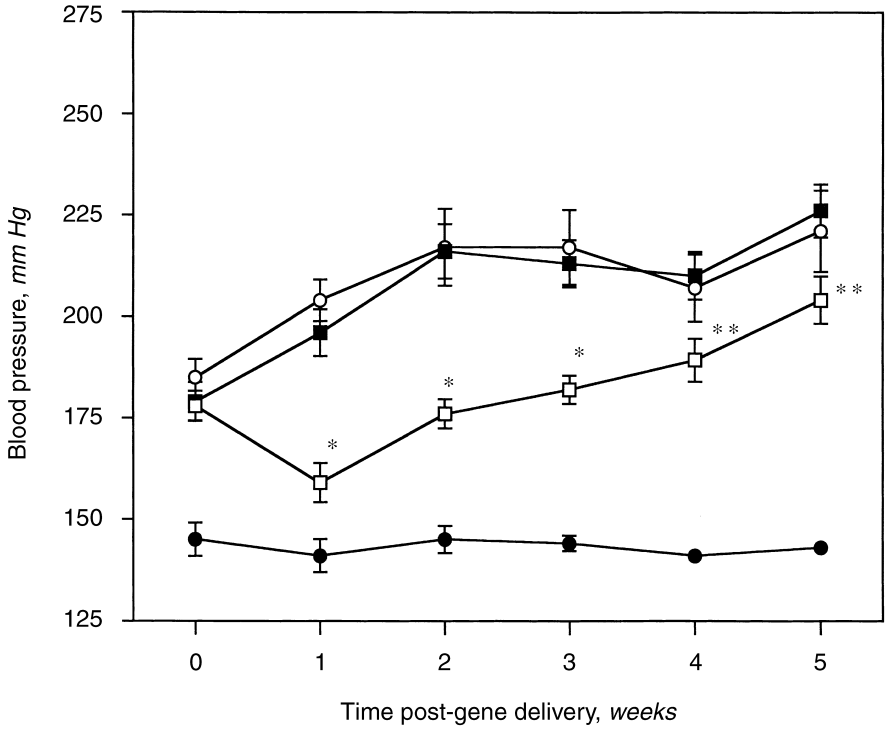


Fig. 1. Systolic blood pressure of sham-operated, 5/6 nephrectomized (reduced renal mass; RRM) rats or nephrectomized rats receiving adenoviral vectors harboring the gene for human tissue kallikrein (cHK) or luciferase (Luc) via tail vein injections. Systolic blood pressure values are expressed as mean ± SEM (N = 6 to 14). Symbols are: (□) cHK; (■) Luc; (○) RRM; (●) sham. *$P < 0.01$ vs. RRM and Luc; **$P < 0.05$ vs. RRM and Luc.

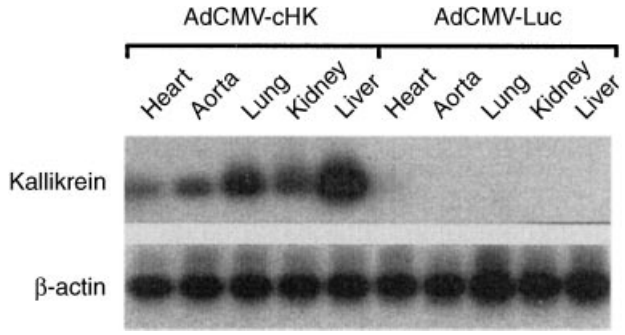


Fig. 2. Expression of human tissue kallikrein mRNA in nephrectomized rats after gene delivery. Total RNA was isolated from heart, aorta, lung, kidney, and liver, and 1 μg was used for RT-PCR, followed by Southern blot analysis.

Improvement in hemodynamic parameters following kallikrein gene delivery

The changes in cardiac index (A), total peripheral vascular resistance (B), regional blood flow to the kidney (C) and gastrocnemius muscle (D) are shown in Figure 4. Kallikrein gene delivery improved cardiac function as evidenced by a significant increase in cardiac index ($P < 0.05$ for cHK compared with Luc, $N = 5$ and 4, respectively) and reduced total peripheral vascular resistance ($P < 0.05$ compared to Luc). A significant increase in regional blood flow to the kidney and gastrocnemius muscle was also found after Ad.CMV-cHK administration ($P < 0.05$ compared with Luc). These results would be consistent with the ability of kinin generation to cause an overall vasodilation in the peripheral vasculature, thereby lowering systemic blood pressure, decreasing vascular resistance and cardiac preload and afterload, and increasing regional blood flow.

Decreased BUN in rats receiving kallikrein gene delivery

Figure 5 shows levels of urea nitrogen in serum of rats over the time course of the study. One week following surgery, BUN levels showed no significant difference between rats with surgery alone and those assigned to receive adenovirus, denoting a uniform effect of surgery. Following gene delivery, rats receiving Ad.CMV-cHK showed significantly decreased BUN levels, indicating enhanced renal function as compared with the control nephrectomized rats (RRM and Luc).

Kallikrein gene delivery attenuates renal injury

Table 1 shows that five-weeks after gene delivery, the group receiving kallikrein gene delivery had significantly less proteinuria than rats with nephrectomy alone ($P < 0.05$) or those receiving luciferase gene delivery ($P < 0.05$). Urinary levels of albumin corroborate these results, as a similar reduction of albuminuria in the cHK group as compared with RRM ($P < 0.05$) and Luc ($P < 0.01$) was seen. Quantitation of glomerular sclerosis in nephrectomized rats five weeks after gene delivery revealed a significant decrease in glomerular sclerotic injury in

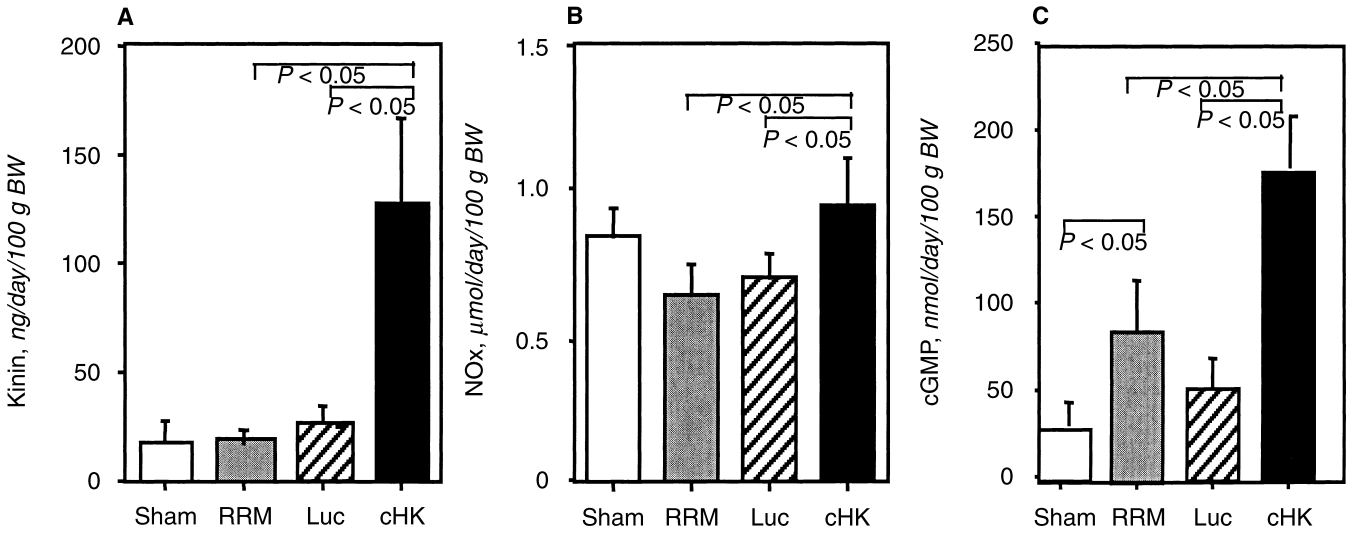


Fig. 3. Urinary excretion of kinin (*A*), nitric oxide (NOx; *B*), and cGMP (*C*) one week following gene delivery. Groups include sham operated (Sham), nephrectomy alone (RRM), and nephrectomized animals receiving gene delivery of human tissue kallikrein (cHK) or luciferase (Luc). Data are expressed as mean ± SEM (N = 6 to 8 per group).

rats receiving kallikrein as compared with RRM ($P < 0.05$) and Luc ($P < 0.05$).

Figure 6 shows kidney sections stained with PAS (renal cortex; row A) or Masson's trichrome (renal medulla; row B) to illustrate the beneficial effects of kallikrein delivery on renal morphology in nephrectomized rats. PAS staining of carbohydrates enables examination of tubular brush borders, basement membranes, and glomerular sclerosis. Normal morphology was seen in the cortex of sham-operated rats (left panel). The renal cortex of nephrectomized rats receiving luciferase gene delivery showed a number of injuries, including proximal tubular damage and loss of brush border, tubular dilation, glomerular sclerosis, the formation of protein casts in tubules, and interstitial inflammation (middle panel). These injuries were also typical in sections of kidney from the RRM group (data not shown), which morphologically did not differ from the luciferase group. Kallikrein gene delivery in nephrectomized rats reduced tubular dilation, brush border disruption, glomerular sclerosis, and interstitial inflammation. Protein casts were reduced both in number and size in the kallikrein group.

In trichrome-stained sections of the medulla (Fig. 6, row B) of nephrectomized rats in the luciferase group, a severe progressive interstitial inflammation and tubular degeneration were evident. Morphologically RRM rats (data not shown) did not differ from the rats receiving luciferase. An increased level of extracellular matrix such as collagen, which stains blue, was evident as compared with the tissue kallikrein group, indicating a greater level of fibrosis. The number of tubules was quite noticeably fewer per field in the luciferase and RRM groups (data not shown) as compared with shams, and the kallikrein group was intermediate.

Kallikrein attenuates cardiac hypertrophy and fibrosis

Figure 7 shows a typical histologic appearance of Masson's trichrome-stained cross-sections of the left ventricle. Masson's trichrome stains cells red and extracellular matrix, such as collagen, blue. The sham-operated hearts appeared morphologically normal. Blue areas of focal fibrosis, like that shown in the middle micrograph in Figure 7, were routinely seen in nephrectomized rats of the RRM (data not shown) and Ad.CMV-Luc groups. Increased blue in interstitial areas was also noted. Blue staining and the size and number of focal areas of fibrosis were reduced in rats receiving kallikrein gene delivery.

Quantitation of blue stain as a percentage of the total tissue area was used as an indicator of left ventricular fibrosis. A significant decrease in blue staining of extracellular matrix, as indicated in Table 1, was evident in the cHK (N = 6) group as compared with RRM ($P < 0.001$, N = 6) and Luc ($P < 0.001$, N = 4) groups. Two complete cross-sections of ventricle were evaluated per animal for a minimum of eight sections per group.

Table 1 also shows the significantly increased left ventricular weight in rats with nephrectomy alone or nephrectomy and luciferase gene delivery as compared with sham-operated rats or nephrectomized rats receiving kallikrein gene delivery ($P < 0.05$, N = 4 to 6 per group). Kallikrein gene delivery also reduced the increase in cardiomyocyte diameter resulting from nephrectomy ($P < 0.01$ for RRM and for RRM + Luc as compared with sham or cHK). Forty cardiomyocytes per animal were measured for a

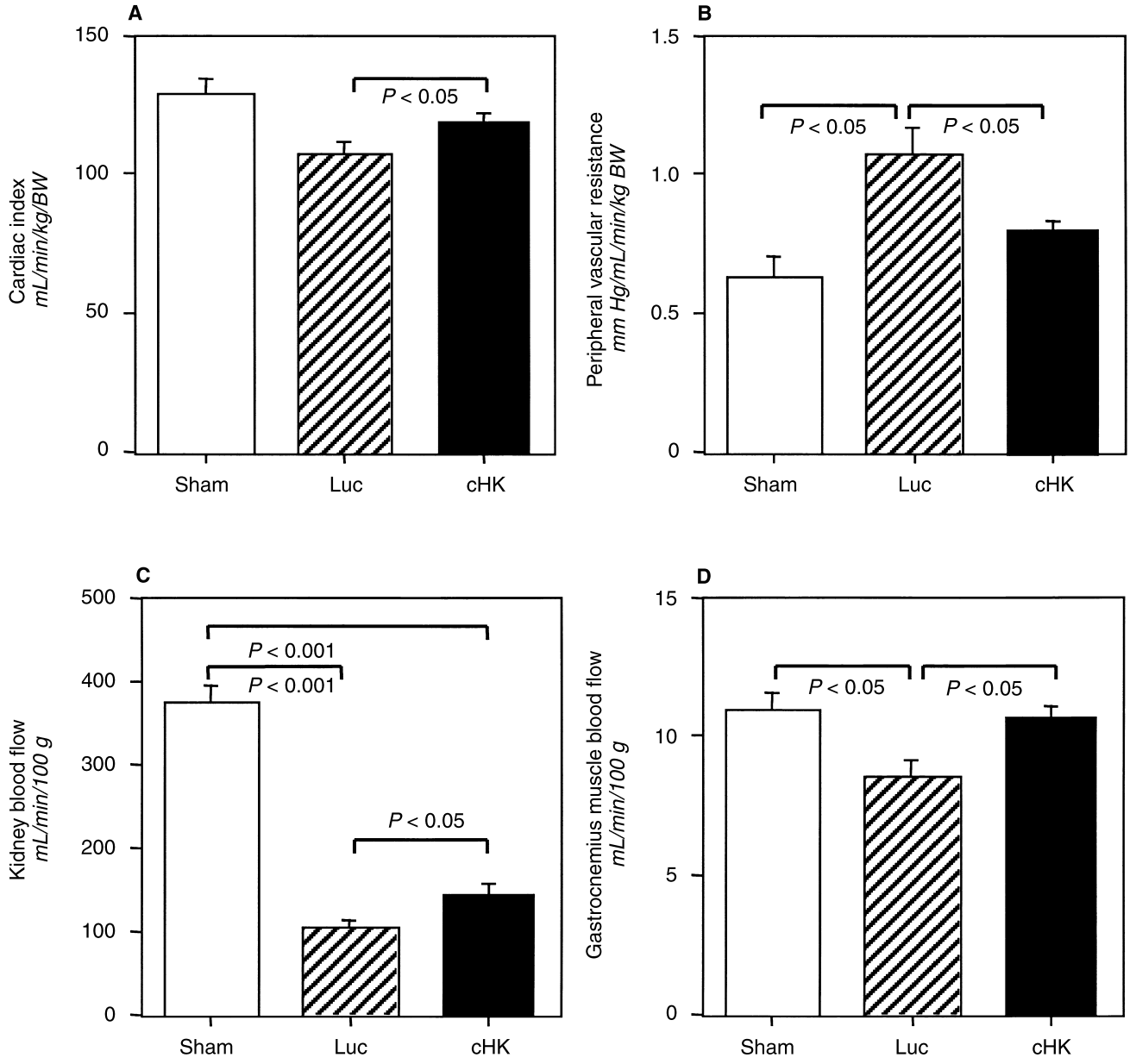


Fig. 4. Hemodynamic parameters measured by fluorescent microspheres one week after gene delivery. Animals include sham-operated controls (sham) or animals that underwent 5/6 nephrectomy received and one week later adenovirus mediated gene delivery for tissue kallikrein (cHK) or the luciferase control virus (Luc). Data are expressed as mean ± SEM (N = 4 to 5).

total of at least 160 per group. The decrease in left ventricular weight and average cardiomyocyte diameter indicate that kallikrein gene delivery attenuates the development of cardiac hypertrophy.

DISCUSSION

This study shows that a single intravenous injection of recombinant adenovirus harboring the human tissue kallikrein gene resulted in a sustained reduction of blood pressure and protection against renal injury and cardiac remodeling in 5/6 nephrectomized rats. The blood pressure-lowering effect was achieved within one week and continued for five weeks following kallikrein gene delivery. Tissue kallikrein mRNA was located in the liver as well as in tissues involved in cardiovascular and renal function, such as the heart, kidney, and aorta. High levels of immunoreactive human tissue kallikrein were detected in the serum and urine of rats receiving kallikrein gene transfer, indicating secretion of human kallikrein from the liver and kidney. Morphological analysis of kidney sections demonstrated that somatic gene delivery

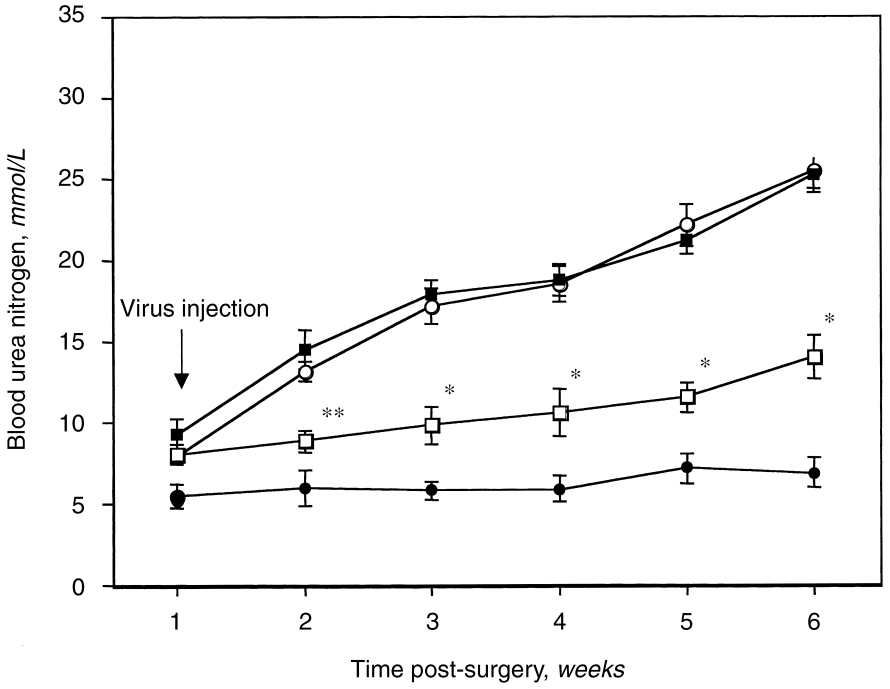


Fig. 5. Time course of BUN levels. Groups include sham operated (●), nephrectomy alone (RRM; ○), and nephrectomized animals receiving gene delivery of human tissue kallikrein (cHK; □) or luciferase (Luc; ■). Data are expressed as mean ± SEM ($N = 4$ or 5 per group). $^{**}P < 0.005$ vs. RRM and Luc; $^{*}P < 0.001$ vs RRM and Luc.

Table 1. Indices of renal and cardiac injury

Parameters	Sham	RRM	Ad.CMV-Luc	Ad.CMV-cHK
Urinary protein *mg/day/100 g body weight*	12 ± 1	76 ± 20	69 ± 15	25 ± 3[a]
Urinary albumin *mg/day/100 g body weight*	0.6 ± 0.1	32.7 ± 8.4	27.1 ± 5.9	9.8 ± 2.3[a]
Glomerular sclerotic score	1.3 ± 1.3	44.8 ± 2.8	41.8 ± 3.7	28.6 ± 4.2[a]
Left ventricle weight/heart weight	0.67 ± 0.01	0.72 ± 0.01	0.72 ± 0.02	0.66 ± 0.01[a]
Myocyte diameter *μm*	17.1 ± 0.24	20.9 ± 0.51	21.0 ± 0.58	19.0 ± 0.68[a]
Left ventricle fibrosis %	0.48 ± 0.02	2.67 ± 0.23	2.72 ± 0.26	0.99 ± 0.10[b]

Measurements are shown as mean ± SEM. All measurements above were made five weeks after gene delivery ($N = 4$ to 6 per group).

[a] $P < 0.05$

[b] $P < 0.001$ as compared with RRM and Ad.CMV-Luc groups

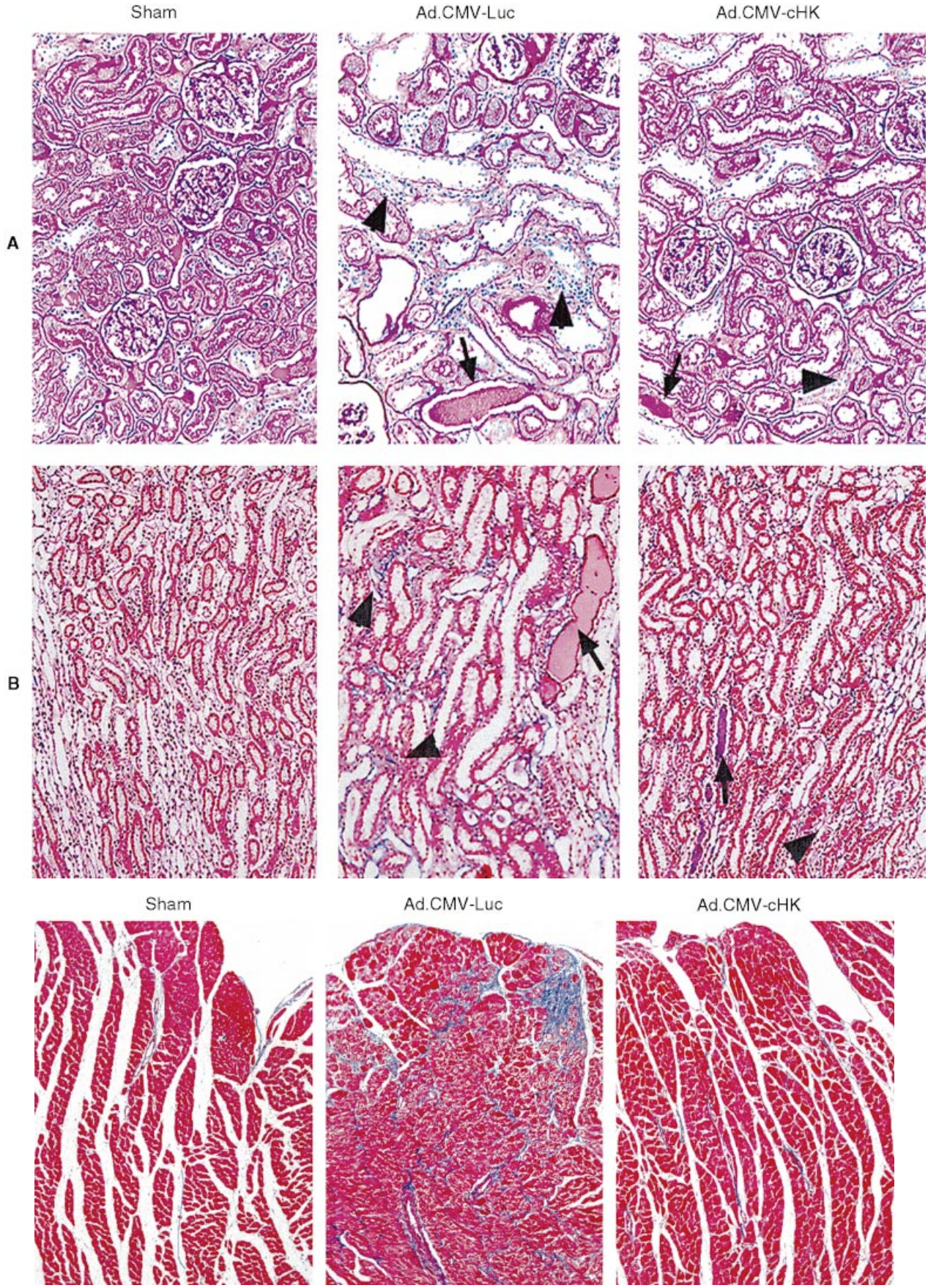
Glomerular lesions were graded using a scale of 0 (normal) to 5 (severely sclerosed), and the final glomerular sclerosis score for each group was calculated from weighted values based on the percentage of glomeruli falling into each severity grade (0 through 5). For left ventricle fibrosis, extracellular matrix was measured in Masson's trichrome-stained sections as the total blue stained area/total tissue area. Groups include sham operated (sham), nephrectomy alone (RRM), and nephrectomized animals receiving gene delivery of human tissue kallikrein (cHK) or luciferase (Luc). Data are expressed as mean ± SEM.

of human tissue kallikrein attenuated glomerular sclerosis, tubulointerstitial inflammation, tubular dilation, brush border disruption, tubular protein cast formation, and reduced proteinuria. Left ventricular hypertrophy and fibrosis were also reduced in rats receiving kallikrein. These findings showed that kallikrein gene delivery has protective effects against cardiovascular remodeling and renal damage in the reduced renal mass model.

Although the therapeutic value of reducing systemic blood pressure in the management of chronic renal disease is well established, locally enhancing the actions of the renal kallikrein-kinin system may also play an

Fig. 6. Representative histologic sections of kidney cortex and medulla. Five weeks after gene delivery, PAS-stained sections of renal cortex (*A*) and Masson's trichrome-stained sections of renal medulla (*B*) were evaluated for renal damage such as thickened basement membranes and glomerular sclerosis, dilated tubules and disrupted brush borders, protein casts (indicated by small arrows) and interstitial inflammation (indicated by larger arrow heads; ×225).

Fig. 7. Representative histologic cross sections of left ventricle stained by Masson's trichrome method. Heart morphology five weeks after gene delivery is presented for sham groups and nephrectomized animals receiving adenovirus harboring human tissue kallikrein (cHK) or luciferase (Luc). Cytoplasm stains red, while extracellular matrix (collagen) stains blue (×200).


Sham
Ad.CMV-Luc
Ad.CMV-cHK
A
B
Sham
Ad.CMV-Luc
Ad.CMV-cHK

important role in the ability of kallikrein gene therapy to slow the progression of renal failure. Kinin has been previously shown to vasodilate glomerular vessels [34, 35], and it has been postulated that by lowering glomerular pressure kinin generation can locally mediate renal protection [33, 36]. Angiotension-converting enzyme (ACE) inhibitors are especially effective in the management of chronic renal failure patients. A number of studies using ACE inhibitors in combination with the highly specific bradykinin B_2 antagonist icatabant have implicated that kinin, at least in part, mediates the beneficial effect of ACE inhibition in cardiovascular disease [37]. In the present study, the roles in renal protection of systemic blood pressure reduction by kallikrein compared with locally mediated renal effects cannot be dissected. However, recent studies using Dahl salt-sensitive hypertensive rats indicated that tissue kallikrein infusion, at levels that did not affect systemic blood pressure, significantly attenuated proteinuria and glomerular injury [33, 36, 38]. Bradykinin B_2 receptor blockade by icatabant was done concomitantly with kallikrein administration in these studies and abrogated the beneficial effect, demonstrating the role of the B_2 receptor in renal protection.

The mechanisms of blood pressure reduction and renal protection after kallikrein gene delivery appear to be mediated by kinin via a NOx-cGMP signal transduction pathway. It is well known that the binding of kinin to endothelial bradykinin B_2 receptors causes production of NO, which subsequently acts as a diffusable second messenger to relax vascular smooth muscle by an increase in cGMP. In 5/6 nephrectomized animals receiving the kallikrein gene, the mean levels of urinary kinin, NOx, and cGMP were increased, indicating the activation of renal bradykinin B_2 receptors occurred with probable subsequent production of NO leading to increased cGMP in the kidney. Previous characterization of hemodynamics in nephrectomized rats demonstrated an increase in systemic blood pressure and a decrease in cardiac index of explanted hearts compared with sham-operated rats [39, 40]. Our analysis of hemodynamics by the microsphere method indicated kallikrein expression caused a relaxation of vascular beds, resulting in an overall decreased peripheral vascular resistance and increased cardiac output and regional flow. These results are consistent with increased kinin production by tissue kallikrein, resulting in activation of endothelial bradykinin B_2 receptors in resistance vessels throughout the peripheral vasculature.

Although adenoviral gene delivery results in high efficiency expression, the lack of viral integration leads to eventual degradation of injected DNA in the cells. In addition to transient recombinant gene expression, the host immune response contributes to problems of inflammation, destruction of host cells, immune reactions to foreign proteins, and thus the ineffectiveness of vector readministration. Recently, modified adenoviral vectors have been shown to produce prolonged transgene expression with markedly reduced immune and inflammatory responses [41]. Therefore, the development of improved adenoviral vectors harboring the human tissue kallikrein gene potentially could be used for studying the role of kallikrein in cardiovascular and renal functions as well as establishing long-term therapeutic potential.

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REFERENCES

1. Clements JA: The glandular kallikrein family of enzymes: Tissue-specific expression and hormonal regulation. *Endocr Rev* 10:393–419, 1989
2. Wang DZ, Song Q, Chen LM, Chao L, Chao J: Expression and cellular localization of tissue kallikrein-kinin system in human adrenal gland. *Am J Physiol* 271:F709–F716, 1996
3. Wolf WC, Harley RA, Sluce D, Chao L, Chao J: Localization and expression of tissue kallikrein and kallistatin in human blood vessels. *J Histochem Cytochem* 47:221–228, 1999
4. Bhoola KD, Figueroa CD, Worthy K: Bioregulation of kinins: Kallikreins, kininogens, and kininases. *Pharmocol Rev* 44:1–80, 1992
5. Hornig D, Drexler H: Endothelial function and bradykinin in humans. *Drugs* 54:42–47, 1997
6. Margolius HS, Horwitz D, Pisano JJ, Keiser HR: Urinary kallikrein excretion in hypertensive man: Relationships to sodium intake and sodium-retaining steroids. *Circ Res* 35:820–825, 1974
7. Margolius HS, Geller R, De Jong W, Pisano JJ, Sjoerdsma A: Altered urinary kallikrein excretion in rats with hypertension. *Circ Res* 30:358–362, 1972
8. Ader JL, Pollock DM, Butterfield MI, Arendshorst WJ: Abnormalities in kallikrein excretion in spontaneously hypertensive rats. *Am J Physiol* 258:F396–F403, 1985
9. Berry TD, Hasstedt SJ, Hunt SC, Wu LL, Smith JB, Ash KO, Kuida H, Williams RR: A gene for high urinary kallikrein may protect against hypertension in Utah kindreds. *Hypertension* 13: 3–8, 1989
10. Wang J, Xiong W, Yang Z, Davis T, Dewey MJ, Chao J, Chao L: Human tissue kallikrein induces hypotension in transgenic mice. *Hypertension* 23:236–243, 1994
11. Song Q, Chao J, Chao L: High level expression of human tissue kallikrein in the circulation induces hypotension in transgenic mice. *Immunopharmacology* 32:105–107, 1996
12. Wang DZ, Chao L, Chao J: Hypotension in transgenic mice overexpressing human bradykinin b-2 receptor. *Hypertension* 29:488–493, 1997
13. Jin L, Zhang JJ, Chao L, Chao J: Gene therapy in hypertension: Adenovirus-mediated kallikrein gene delivery in hypertensive rats. *Hum Gene Ther* 8:1753–1761, 1997
14. Chao J, Zhang JJ, Lin KF, Chao L: Human kallikrein gene delivery attenuates hypertension, cardiac hypertrophy, and renal injury in Dahl salt-sensitive rats. *Hum Gene Ther* 9:21–31, 1998
15. Yayama K, Wang C, Chao L, Chao J: Kallikrein gene delivery attenuates hypertension and cardiac hypertrophy and enhances renal function in Goldblatt hypertensive rats. *Hypertension* 31:1104–1110, 1998
16. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
17. Anderson S, Rennke HG, Brenner BM: Therapeutic advantage

of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J Clin Invest* 77:1993–2000, 1986
18. Jackson B, Hodsman P, Johnston CI: Changes in the renin-angiotensin system, exchangeable body sodium, and plasma and atrial content of atrial natriuretic factor during evolution of chronic renal failure in the rat. *Am J Hypertens* 1:298–300, 1988
19. Potter GS, Johnson RJ, Fink GD: Role of endothelin in hypertension of experimental chronic renal failure. *Hypertension* 30:1578–1584, 1997
20. Rosenberg ME, Correa-Rotter R, Inagami T, Kren SM, Hostetter TH: Glomerular renin synthesis and storage in the remnant kidney in the rat. *Kidney Int* 40:677–683, 1991
21. Campese VM: Neurogenic factors and hypertension in chronic renal failure. *J Nephrol* 10:184–187, 1997
22. Shimamoto K, Ura N, Ishiguro T, Nakagawa M, Iimura O: The role of renal natriuretic depressor systems on hypertensive mechanisms in reduced renal mass hypertensive rats. *Hypertens Res* 18:S53–S57, 1995
23. Nakamura T, Obata J, Kimura H, Ohno S, Yoshida Y, Kawachi H, Shimizu F: Blocking angiotensin II ameliorates proteinuria and glomerular lesions in progressive mesangioproliferative glomerular nephritis. *Kidney Int* 55:877–899, 1999
24. Nabokov A, Amann K, Gassmann P, Schwarz U, Orth SR: The renoprotective effect of angiotensin-converting enzyme inhibitors in experimental chronic renal failure is not dependent on enhanced kinin activity. *Nephrol Dial Transplant* 13:173–176, 1998
25. Zoja C, Donadelli R, Corna D, Testa D, Facchinetti D, Maffi R, Luzzana E, Colosio V, Bertani T, Remuzzi G: The renoprotective properties of angiotensin-converting enzyme inhibitors in a chronic model of membranous nephropathy are solely due to the inhibition of angiotensin II: Evidence based on comparative studies with a receptor antagonist. *Am J Kidney Dis* 29:254–264, 1997
26. MacLaughlin M, Monserrat AJ, Muller A, Matoso M, Amorena C: Role of kinins in the renoprotective effect of angiotensin-converting enzyme inhibitors in experimental chronic renal failure. *Kidney Blood Press Res* 21:329–334, 1998
27. Floege J, Burns MW, Alpers CE, Yoshimura A, Pritzl P, Gordon K, Seifert RA, Bowen-Pope DF, Couser WG, Johnson RJ: Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Int* 41:297–309, 1992
28. Bauer J: *Clinical Laboratory Methods* (9th ed). St. Louis, The C.V. Mosby Company, 1982
29. Silver A, Dawnay A, Landon J, Cattell WR: Immunoassays for low concentrations of albumin in urine. *Clin Chem* 32:1303–1306, 1986
30. Brooker G, Harper JF, Terasaki WL, Moylan RD: Radioimmunoassay of cyclic AMP and cyclic GMP. *Adv Cyclic Nucleic Res* 10:1–33, 1979
31. Misko TP, Schilling RJ, Salvemini D, Moore WM, Currie MG: A fluorometric assay for the measurement of nitrite in biological samples. *Anal Biochem* 214:11–16, 1993
32. Klemm K, Moody FG: Regional intestinal blood flow and nitric oxide synthase inhibition during sepsis in the rat. *Ann Surg* 227:126–133, 1998
33. Uehara Y, Hirawa N, Kawabata Y, Suzuki T, Ohshima N, Oka K, Ikeda T, Goto A, Toyo-oka T, Kizuki K, Omata M: Long-term infusion of kallikrein attenuates renal injury in Dahl salt-sensitive rats. *Hypertension* 24:770–778, 1994
34. Arima S, Ito S, Omata K, Takeuchi K, Abe K: High glucose augments angiotensin II action by inhibiting NO synthesis in in vitro microperfused rabbit afferent arterioles. *Kidney Int* 48:683–689, 1995
35. Heller J, Kramer HJ, Horacek V: Comparative effects of the angiotensin II receptor blocker EXP 3174 and of the angiotensin-converting enzyme inhibitor captopril on renal glomerular hemodynamics in the dog. *Kidney Blood Press Res* 20:391–397, 1997
36. Hirawa N, Uehara Y, Suzuki T, Kawabata Y, Numabe A, Gomi T, Ikeda T, Kizuki K, Omata M: Regression of glomerular injury by kallikrein infusion in Dahl salt-sensitive rats is a bradykinin B2-receptor-mediated event. *Nephron* 81:183–193, 1999
37. Mombouli JV: ACE inhibition, endothelial function and coronary artery lesions: Role of kinins and nitric oxide. *Drugs* 54:12–22, 1997
38. Uehara Y, Hirawa N, Numabe A, Kawabata Y, Ikeda T, Gomi T, Gotoh A, Omata M: Long-term infusion of kallikrein attenuates renal injury in Dahl salt-sensitive rats. *Am J Hypertens* 10:83S–88S, 1997
39. el-Belbessi S, Brautbar N, Anderson K, Campese VM, Massry SG: Effect of chronic renal failure on heart: Role of secondary hyperparathyroidism. *Am J Nephrol* 6:369–375, 1986
40. Raine AE, Seymour AM, Roberts AF, Radda GK, Ledingham JG: Impairment of cardiac function and energetics in experimental renal failure. *J Clin Invest* 92:2934–2940, 1993
41. Ilan Y, Droguett G, Chowdhury NR, Li Y, Sengupta K, Thummala NR, Davidson A, Chowdhury JR, Horwitz MS: Insertion of the adenoviral E3 region into a recombinant viral vector prevents antiviral humoral and cellular immune responses and permits long-term gene expression. *Proc Natl Acad Sci USA* 94:2587–2592, 1997